recombinant antibodies, and [monoclonal] <u>antigen-binding</u> antibody fragments <u>thereof</u> that specifically bind to a stable, conformation independent epitope <u>of a lipoprotein containing Apo A-I or Apo A-II</u> which is uninfluenced by the lipid content of the lipoprotein, [apolipoprotein] <u>protein component of the lipoprotein,</u> or lipid associated with a specific lipoprotein.

47.   (twice amended) The kit of claim 46 wherein the anti-Apo A-I and anti-Apo A-II monoclonal or recombinant antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, [apolipoprotein] <u>protein component of the lipoprotein,</u> or lipid associated with a specific lipoprotein.

<div align="center"><u>Remarks</u></div>

Rejections under 35 U.S.C. 112

Claim 39 was rejected under 35 U.S.C. 112 as containing subject matter which was not described in the specification. This rejection is respectfully traversed.

The examiner states that "The specification is drawn to apolipoproteins and the claims are drawn to lipoproteins. Applicants mismatch lipoproteins and apolipoproteins....Apolipoproteins are components of lipoproteins and the concept of the specification are for non-cross reactive antibodies are for apoliproteins and not

<div align="center">11</div>

lipoproteins as asserted". Applicants agree that apolipoproteins and lipoproteins

are not synonymous.

It is well known that lipoproteins contain a protein component and a lipid

component. The protein component in the absence of the lipid is referred to as the

"apolipoprotein". Examples of lipoproteins include high density lipoprotein, or

"HDL", and low density lipoprotein, or "LDL". The antibodies in the application are

reactive with the lipoproteins, since it is the lipoproteins that are present in serum,

the preferred material to be tested for lipoprotein levels. The examiner is incorrect

when she states that the specification is drawn to apolipoproteins, since the

specification discloses both apolipoproteins as well as lipoproteins. The basis of the

invention, however, is antibodies which bind to lipoprotein regardless of the lipid or

protein content, i.e., "an epitope uninfluenced by the lipid content of the lipoprotein,

apolipoprotein or lipid associated with a specific lipoprotein". This is made even

clearer by reference to the examples. **Example 7 shows binding of LDL using**

mAB HB₃cB₃. Example 8 shows binding of HDL by mAb AIbD₅. Page 17,

lines 22-23, state "Mabs that bind to a single apolipoprotein with no significant

detectable crossreactivity with other apolipoproteins are considered specific". Page

17, line 24, to page 18, line 16, describes how to determine if there is crossreactivity.

Page 27, line 33 to page 28, line 10, states that mAB HB₃cB₃ is specific for LDL and

not cross-reactive with other lipoproteins. Page 28, lines 18-25, Tables 1 and 2, state

that mAb AIbD₅ is specific to HDL. Since the antibodies are reactive with the

lipoprotein regardless of the lipid content, it is likely they are reactive with a

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specific epitope on the protein component of the lipoprotein, and therefore bind to the epitope in the absence of any lipid (an apolipoprotein). Therefore the claim is fully supported by the specification.

However, the problem may be that the claim is poorly worded, so the word "apolipoprotein" has been deleted from the last line of the claim and the claim amended to recite the "protein component of the lipoprotein". This makes it clear that the antibody is to the lipoprotein, and the immunoreaction is not influenced by the lipid content (amount or nature) nor by the protein present in the lipoprotein. This same change has been made in other claims for consistency and clarity. This is not to say, however, that the antibodies are not reactive with apolipoprotein components of the lipoprotein.

Claims 1-13, and 40-45 were rejected under 35 U.S.C. 112 as indefinite. This rejection is respectfully traversed if applied to the amended claims. The undersigned has attempted to respond to each concern or implement the proposed changes.

Claim 1 has been amended to clarify that the antibodies are reactive with at least two different lipoproteins or apolipoproteins. Claim 1 has also been amended to recite that the antibodies are selected from the group consisting of monoclonal antibodies, and recombinant antibodies or fragments derived therefrom, to correct antecendent basis for claim 3 and 5, which were rejected as failing to further limit the subject matter of the independent claim.

Claim 6 has been amended as suggested by the examiner.

Claim 9 has been amended to clarify that the stained protein is that associated with the lipid stained in the method of claim 6.

Claims 12 and 13 have been amended to correct the Markush language and to add a clearly defined step in which the relative amounts of the two apolipoproteins are determined

Claim 40 has been amended to clarify the method steps as noted by the examiner.

Claim 41 has been amended to provide antecedent basis as suggested by the examiner.

Claim 42 has been amended to delete the reference to ApoB, to correct the problem noted by the examiner.

With respect to claims 44 and 45, the term "predominantly" is a well defined term understood by those skilled in the art to mean "to be dominant in amount, number, etc." (Webster's new World Dictionary). This means at least more than one-half. No greater specificity is required.

Rejections under 35 U.S.C. 103

Claims 1, 10 and 11 were rejected under 35 U.S.C. 103 as obvious over U.S. Patent No. 5,126,276 to Fish, et al., in combination with EP 0262854 to Scripps and Forster, et al., Biochem. Soc. Trans. 18(6):1180(1990) Zhow, et al., Hubi Yixueyuan Xueabo. II(4), 298-302 (1998) and Koren, et al. Atheroscloerosis 95, 157-170 (1992), alone or in combination with U.S. Patent No. 4,677,057 to Curtiss. Claim 6 was rejected under 103 as obvious over Fish in combination with Scripps, Forster, and

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Zhow, and Koren, et al.. Claims 7 and 8 were rejected under 103 as obvious over

Fish, Scripps, Forster, and Zhow, Koren and further in combination with Mills, et

al. Laboratory Techniques in biochemistry and molecular biology, vol. 14, pages

472-478 (1984). Claims 12 and 1 were rejected under 103 as obvious over Fish,

Scripps, Forster, Zhow, Koren and EO 0 257 778 by Scripps. Claims 42-45 were

rejected under 35 U.S.C. 103 as obvious over Koren, et al., (1992). These rejections

are respectfully traversed.

The basis for these rejections are that the language "or apolipoproteins"

broadens the claims so that they read on the prior art, since there is no longer a

requirement that the antibody binds to a lipoprotein in a manner that is

uninfluenced by lipid associated with the specific lipoprotein. This is not correct.

Claim 1 recites "wherein the antibody molecules are selected from the group

consisting of monoclonal antibodies, recombinant antibodies and antigen-binding

antibody fragments thereof, wherein the antibody molecules are immunoreactive

with at least two different lipoproteins, wherein the first and second antibodies bind

to different stable, conformation independent epitopes that are uninfluenced by the

lipid content of the lipoprotein, protein component of the lipoprotein or lipid

associated with the specific lipoprotein, wherein the lipoproteins are selected from

the group consisting of LDL, HDL and VLDL"

This limitation does not preclude reaction of the antibody with a protein

component of the lipoprotein, however, it does strictly limit the antibodies to be

used in the method as claimed. None of the prior art discloses such an antibody.

Rejections of claims 1, 10 and 11 over Fish, Scripps, Forster, Zhow,

Koren and Curtiss

Fish shows substrates suitable for use of immobilized antibodies that can be

dipped into a sample solution to bind to antigen. There is no disclosure of using

multiple antibodies immunoreactive with LDL, HDL or VLDL on the **same**

substrate to provide a comparative ratio. Therefore Fish does not disclose the

claimed method. The remaining art does not make up for this deficiency.

Scripps describes assays in which the binding of Apo-B100 relative to the

amount of ApoAI is compared. Not only is there no disclosure of antibodies that

allow collection of comparative data, there is also no disclosure of antibodies which

bind to different stable, conformation independent epitopes that are uninfluenced

by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the

specific lipoprotein. As described at page 13, lines 10-14, the antibodies to Apo B100

reacted with **all three lipoproteins**, LDL, VLDL and IDL. As described at page

17, lines 10-20, binding of lipid (as in chylomicrons) affects binding to Apo B100.

The data at page 18, lines 12-18, indicates that binding of the anti-Apo AI

antibodies is affected by lipid, therefore they are not reactive with stable,

conformation independent epitopes uninfluenced by lipid content. In contrast,

plasma or serum samples can be used undiluted with the conformation and lipid

independent antibodies described by applicant. Therefore the antibodies of claim 1

and claims dependent thereon are not disclosed by Scripps. The assay utilized by

Scripps (see page 8, lines 1-26) is very complex as a result of the problems that arise

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as a result. As the examiner is aware from the prosecution of applicants' related applications, it was applicants' development of a technique to produce antibodies that bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein that was critical to development of the claimed assays and kits for use therein.

Forster describes the desirability of a two antibody assay to obtain the ratio of HDL to LDL but provides no details as to how such an assay could be done or what antibodies are used.

Zhow merely demonstrates that ratios of Apo AI to Apo B may be useful in the diagnosis of heart disease.

Assuming one were motivated to determine the ratio of Apo AI to Apo B, one would still not have the claimed assay. The prior art antibodies fail to completely distinguish between VLDL, LDL and HDL, or the individual apolipoproteins under conditions of varying lipid concentration or conformation. The claims have been amended to clarify and more clearly define these difference.

As noted on page 29, lines 3-13, Koren (1992) describes antibodies to Apo CIII and Apo E. These are not described as antibodies that bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein, and do not completely distinguish between lipoproteins (note the use of the modifying term

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"predominantly"). Even in combination with the other prior art, one would not achieve the claimed assays.

Curtiss describes four different monoclonal antibodies. These do not bind the same epitopes, but they also do not bind to the epitopes equally well in different lipoprotein populations. Curtiss even states at col. 14, lines 58-60, "There is no reason to assume that conformational variation will be identical for lipid-free and lipid-associated apo-A-I." Curtiss therefore recognizes the problem (the use of antibodies in an assay is affected by the conformation and lipid content of the lipoprotein) but chooses another approach to solve it. There is nothing that would lead one to conclude that the solution would be to obtain antibodies that bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein, much less how to go about achieving such a goal.

Claim 6 is not obvious from Fish, Scripps, Forster, Zhow, Koren and Lucas

The other references are discussed above. Claim 6 adds the further element that the lipid bound to the immobilized antibodies is stained with a lipid stain.

Lucas does not make up for the failure of the other art to provide antibodies that bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein deficiency. Lucas also recognizes the desirability of determining the relative amounts of HDL and LDL, as well as

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apolipoproteins. The Lucas assay requires immobilization of the intact lipoproteins so that the other sample components can be removed. Lucas says (col. 10, lines 9-12) that monoclonals are better for recognition of specific epitopes. The need to remove other sample components clearly indicates that the other sample components would have an effect on the assay as Lucas envisions it, so the antibodies cannot be antibodies that bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with the specific lipoprotein. Moreover, based on the disclosure at col. 16, lines 51-60, the immunogens are not treated to delipidate and solubilize and reduce the molecules so that one could obtain antibodies that are reactive with lipid and conformation independent epitopes.

Claims 7 and 8 are not obvious over Fish, Scripps, Forster, Zhow, Koren, Luca and Mills

The other prior art is discussed above. Claim 7 requires staining with specific dyes. Claim 8 requires staining before immersion of the immobilized antibody into the sample.

Mills does teach staining of lipids. However, as discussed above, this would still not lead those skilled in the art to the claimed methods and kits for use therein. The prior art simply fails to provide a means whereby one can place different antibodies on the same substrate to differentiate, measure and compare two different lipoproteins at the same time.

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Claims 12 and 13 are not obvious over Fish, Scripps, Forster, Zhow, Koren and Scripps

The art has been discussed above. The examiner's position is that the combination differs by not combining antibody with the sample prior to immersion of the substrate containing the immobilized antibody into the sample. However, this goes to the very heart of the difference between the prior art and what is claimed: it is the differences in the specificity of the antibodies that allows one to differentiate between lipoproteins and therefore to use a single substrate having antibodies immobilized thereon to detect multiple different antigens, and the prior art fails to teach such antibodies: antibodies that bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, protein component thereof or lipid associated with the specific lipoprotein, as claimed.

Allowance of claims 1-13 and claims 39-47 is earnestly solicited.

Respectfully submitted,
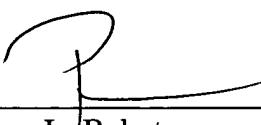
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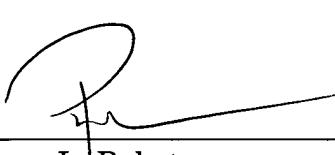
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Patrea L. Pabst

APPENDIX: Clean copy of amendment to specification

This application is a continuation of U.S. Serial No. 08/268,809 filed June 30,

1994, now U.S. Patent No. 6,107,045.

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APPENDIX: **Marked up Copy of Claims as Amended Upon Entry of the Amendment**

1.   (four times amended) A method for determining the relative ratio of at least two different <u>lipoproteins or</u> apolipoproteins in a biological sample comprising:

immersing into the sample a solid phase material having separately immobilized thereon at least first and second [monoclonal] antibody molecules, <u>wherein the antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies and antigen-binding antibody fragments thereof, wherein the antibody molecules are</u> immunoreactive with [LDL, HDL or VLDL or] at least two different <u>lipoproteins</u> [apolipoproteins], wherein the first and second [monoclonal] antibodies bind to different stable, conformation independent epitopes that are uninfluenced by the lipid content of the lipoprotein, [apolipoprotein] <u>protein component of the lipoprotein</u> or lipid associated with the specific lipoprotein, wherein the lipoproteins are selected from the group consisting of LDL, HDL and VLDL;

allowing the [monoclonal] antibody molecules time to bind to the LDL, HDL, [or] VLDL or apolipoproteins in the sample;

removing the solid phase material containing the immobilized [monoclonal] antibody molecules;

determining the amount of [LDL, HDL or VLDL] lipoprotein or [at least two different] apolipoproteins bound by the immobilized[monoclonal] antibody molecules, and

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comparing the amount bound which is specific for LDL, HDL_x [or] VLDL or

each apolipoprotein in order to calculate the relative amounts of LDL, HDL_x [or]

VLDL or apolipoproteins.

2.   (amended) The method of claim 1 wherein the antibody molecules

immobilized on the solid phase material are immunoreactive with lipoproteins

selected from the group consisting of HDL and LDL.

3.   (twice amended) The method of claim 2 wherein the antibodies to the

HDL or LDL are selected from the group consisting of recombinant antibodies and

antibody fragments.

4.   (twice amended) The method of claim 3, wherein the first or second

monoclonal antibodies are the anti-LDL monoclonal antibody produced by the

hybridoma cell line HB_3cB_3 ATCC designation number HB 11612.

5.   (twice amended) The method of claim 3, wherein the first or second

monoclonal antibodies are recombinant anti-LDL $RcB_3M_1D_4$ ATCC designation

number 69602.

6.   (three times amended) The method of claim 1 further comprising

determining the amount of lipoprotein lipid or lipid associating with apolipoprotein

by staining of the material bound to the immobilized antibody using a lipid stain.

7.   The method of claim 6 wherein the lipid stain is selected from the

group consisting of Sudan Red 7B, Oil Red O, and Sudan Black B.

8.   The method of claim 6 wherein the lipoprotein lipid is stained prior to

immersing the immobilized antibodies.

9.   (three times amended) The method of claim 6 further comprising

measuring the amount of apolipoprotein or protein associated with the lipid in the

sample, further comprising the step of providing antibodies immunoreactive with at

least one [the] apolipoprotein, wherein the antibodies are coupled to a protein stain,

and staining the apolipoprotein or protein associated with the lipid in the sample by

reacting the protein stain coupled antibodies with the apolipoprotein or protein

associated with the lipid in the sample.

10.   The method of claim 1, wherein the apolipoprotein is selected from

the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

11.   The method of claim 1, wherein the biological sample is selected from

the group consisting of blood, plasma, and serum.

12.   (four times amended) A method of determining the relative

concentration of at least two different apolipoproteins in a biological sample

comprising:

mixing in solution a first and second monoclonal antibody molecules each

immunoreactive with a specific different apolipoprotein into the sample, wherein at

least one of the first and second monoclonal antibodies bind to a stable,

conformation independent epitope of a lipoprotein that is uninfluenced by the lipid

content of [a] the lipoprotein, [apolipoprotein] protein component of the lipoprotein

or lipid associated with [a] the specific lipoprotein [selected from the group

consisting of different apolipoproteins] in a conformation and lipid content

independent manner;

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allowing the monoclonal antibody molecules to bind to the [apolipoprotein] apolipoproteins in the sample,

immersing into the mixture third immobilized monoclonal antibody molecules immunoreactive with a second, distinct epitope of one of the first or second apolipoproteins,

allowing the third immobilized monoclonal antibody molecules to bind to one of the apolipoproteins bound by either the first or second monoclonal antibodies,

[detecting the presence of the apolipoprotein bound by either the first or second monoclonal antibodies and the third immobilized monoclonal antibodies, and]

determining the amount of apolipoprotein bound by [either] the first [or] and second monoclonal antibodies and the amount of protein bound by the third immobilized monoclonal antibodies, and

subtracting from the total apolipoprotein bound by the first and second monoclonal antibodies the amount of protein bound by the third immobilized monoclonal antibodies, to yield the amounts of the first and second apolipoproteins .

13.   (amended) The method of claim 12 wherein the apolipoprotein bound by one of the monoclonal antibodies in solution is apolipoprotein Apo B-100.

39.   (three times amended) A method for determining the relative ratio of LDL to HDL in a biological sample comprising

(a) determining the amount of LDL in the sample by

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adding to the sample monoclonal antibody molecules immunoreactive with low density lipoprotein and not cross-reactive with high density lipoprotein and determining the amount of low density lipoprotein;

(b) determining the amount of HDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with high density lipoprotein and not cross-reactive with low density lipoprotein and determining the amount of high density lipoprotein; and

(c) determining the ratio of the amount of low density lipoprotein with the amount of high density lipoprotein, wherein at least one of the monoclonal antibodies to LDL and HDL bind a stable, conformation independent epitope that is uninfluenced by the lipid content of the lipoprotein, <u>the protein component of the lipoprotein</u> [apolipoprotein] or lipid associated with the specific lipoprotein.

40.   (twice amended) A method for determining the relative ratio of VLDL to HDL in a biological sample comprising

(a) determining the amount of VLDL in the sample by

determining the amount of Apo C-III present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

contacting the anti-ApoC-III antibody reactive with Apo C-III with the biological sample to form complexes between the anti-ApoC-III antibody and the Apo C-III containing lipoprotein particles,

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contacting the Pan B antibody with the biological sample containing the anti-

ApoC-III antibody bound to the Apo C-III containing lipoprotein particles,

separating the complexed Pan B-anti-ApoC-III antibody-lipoprotein particles

from the biological sample, <u>and</u>

<u>determining the amount of complexed Pan B-anti-ApoC-III antibody-</u>

<u>lipoprotein particles,</u> which is the amount of Apo C-III present in VLDL in the anti-

Apo C-III anti-Apo B complexed material in the sample;

and

(b) determining the amount of HDL in the sample by

determining the amount of Apo C-III present in the HDL in the sample by

providing Apo A-I monoclonal antibody specifically immunoreactive with Apo

A-I,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

contacting the antibody reactive with Apo C-III with the biological sample to

form complexes between the anti-Apo C-III antibody and the Apo C-III containing

lipoprotein particles,

contacting the anti-Apo A-I antibody with the biological sample to form

complexes with the anti-Apo C-III antibody-Apo C-III containing lipoprotein

particles,

separating the complexed anti-Apo C-III antibody-Apo C-III containing

lipoprotein particles from the biological sample,

determining the amount of Apo C-III present in HDL in the anti-Apo C-III-anti-Apo A-I complexed material in the sample, and

determining the ratio of Apo C-III present in VLDL in the sample to Apo C-III present in HDL in the sample, which is the ratio of VLDL to HDL,

wherein the VLDL and HDL are measured in the same sample using immobilized anti-Apo A-I and anti-Apo B or anti-Apo C-III antibodies or measured by immunoprecipitation with the anti-Apo A-I and anti-ApoB antibodies or anti-Apo C-III antibodies in separate samples,

wherein at least one of the monoclonal antibodies bind to a stable, conformation independent epitope that is uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with a specific lipoprotein selected from the group consisting of Apo AI, Apo B, and Apo CIII.

41.   (three times amended) A method for determining the relative ratio of VLDL to HDL comprising

(a) determining the amount of VLDL in the sample by

determining the amount of Apo E present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody which specifically binds to Apo E associated with VLDL,

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contacting the antibodies reactive with Apo E associated with VLDL with the

biological sample to form complexes between the anti-ApoE antibodies and Apo E

containing particles,

contacting Pan B antibody with the biological sample containing the

complexes between the anti-ApoE antibodies and ApoE containing particles to form

complexes of anti-ApoB-anti-ApoE-ApoE containing particles, and

determining the amount of Apo E in the complexes of anti-ApoB-anti-ApoE-

ApoE containing particles, which is the Apo E present in VLDL in the sample;

(b) removing the complexes of anti-ApoB-anti-ApoE-ApoE containing

particles, either by binding of the anti-Apo E antibodies to an immobilized surface

or centrifugation of sample to remove the complexes of anti-ApoB-anti-ApoE-ApoE

containing particles;

and

(c) determining the amount of HDL in the sample by

determining the amount of Apo E present in the HDL in the sample by

providing Apo A-I monoclonal antibody immunoreactive specifically with Apo

A-I,

contacting [the] antibodies reactive with Apo E in HDL particles with the

biological sample to form complexes between the anti-ApoE antibodies and Apo E

containing particles,

contacting the Apo A-I monoclonal antibody with the biological sample to

form complexes of the anti-ApoE antibodies-ApoE containing particles-anti-ApoA-I,

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determining the amount of Apo E present in HDL in the complexes of the

anti-ApoE antibodies-ApoE containing particles-anti-Apo A-I in the sample, and

determining the ratio of Apo E present in VLDL in the sample and Apo E

present in HDL in the sample which is the ratio of VLDL to HDL,

wherein at least one of the monoclonal antibodies bind to a stable,

conformation independent epitope that is uninfluenced by the lipid content of the

lipoprotein, [apolipoprotein] protein component of the lipoprotein or lipid associated

with a specific lipoprotein selected from the group consisting of Apo B, Apo AI, and

Apo E.

42.   (three times amended) A kit for determining the relative ratio of

VLDL to HDL comprising

Pan B antibody which is characterized by an equal binding and high affinity

for all Apo B-containing lipoproteins in human plasma,

monoclonal or recombinant antibody specifically immunoreactive with Apo C-

III, and

monoclonal or recombinant Apo A-I antibody specifically immunoreactive

with Apo A-I,

wherein at least one of the monoclonal or recombinant antibodies specifically

bind to a stable, conformation independent epitope of a lipoprotein including Apo C-

III or Apo A-I that is uninfluenced by the lipid content of the lipoprotein,

[apolipoprotein] protein component thereof or lipid associated with a specific

lipoprotein selected from the group consisting of [Apo B,] Apo AI, and Apo CIII.

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43.   (twice amended) The kit of claim 42 wherein the anti-Apo C-III or anti-A-1 monoclonal or recombinant antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and [monoclonal] antigen binding antibody fragments thereof that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, [apolipoprotein] protein component thereof, or lipid associated with a specific lipoprotein.

44.   (twice amended) A kit for determining the relative ratio of VLDL to HDL comprising

Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

monoclonal antibody which predominantly binds to Apo E associated with VLDL ,

monoclonal Apo A-I antibody specifically immunoreactive with Apo A-I, and

monoclonal antibody which predominantly binds to Apo E in HDL,

wherein at least one of the antibodies binds to a stable, conformation independent epitope of a lipoprotein containing Apo E or Apo A-I that is uninfluenced by the lipid content of the lipoprotein, [apolipoprotein] protein component of the lipoprotein or lipid associated with a specific lipoprotein.

46.   (twice amended) A kit for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

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monoclonal or recombinant Apo-A-I antibody specifically immunoreactive

with Apo A-I lipoproteins in human plasma; and

monoclonal or recombinant Apo A-II antibody specifically immunoreactive

with Apo A-II,

wherein the anti-Apo A-I or anti-Apo A-II monoclonal or recombinant

antibody molecules are selected from the group consisting of monoclonal antibodies,

recombinant antibodies, and [monoclonal] antigen-binding antibody fragments

thereof that specifically bind to a stable, conformation independent epitope of a

lipoprotein containing Apo A-I or Apo A-II which is uninfluenced by the lipid

content of the lipoprotein, [apolipoprotein] protein component of the lipoprotein, or

lipid associated with a specific lipoprotein.

47.   (twice amended) The kit of claim 46 wherein the anti-Apo A-I and

anti-Apo A-II monoclonal or recombinant antibody molecules are selected from the

group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal

antibody fragments that specifically bind to a stable, conformation independent

epitope which is uninfluenced by the lipid content of the lipoprotein,

[apolipoprotein] protein component of the lipoprotein, or lipid associated with a

specific lipoprotein.

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comparing the amount bound which is specific for LDL, HDL, VLDL or each apolipoprotein in order to calculate the relative amounts of LDL, HDL, VLDL or apolipoproteins.

2.   (amended) The method of claim 1 wherein the antibody molecules immobilized on the solid phase material are immunoreactive with lipoproteins selected from the group consisting of HDL and LDL.

3.   (twice amended) The method of claim 2 wherein the antibodies to the HDL or LDL are selected from the group consisting of recombinant antibodies and antibody fragments.

4.   (twice amended) The method of claim 3, wherein the first or second monoclonal antibodies are the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3 ATCC designation number HB 11612.

5.   (twice amended) The method of claim 3, wherein the first or second monoclonal antibodies are recombinant anti-LDL $RcB_3M_1D_4$ ATCC designation number 69602.

6.   (three times amended) The method of claim 1 further comprising determining the amount of lipoprotein lipid or lipid associating with apolipoprotein by staining of the material bound to the immobilized antibody using a lipid stain.

7.   The method of claim 6 wherein the lipid stain is selected from the group consisting of Sudan Red 7B, Oil Red O, and Sudan Black B.

8.   The method of claim 6 wherein the lipoprotein lipid is stained prior to immersing the immobilized antibodies.

9.   (three times amended) The method of claim 6 further comprising

measuring the amount of apolipoprotein or protein associated with the lipid in the

sample, further comprising the step of providing antibodies immunoreactive with at

least one apolipoprotein, wherein the antibodies are coupled to a protein stain, and

staining the apolipoprotein or protein associated with the lipid in the sample by

reacting the protein stain coupled antibodies with the apolipoprotein or protein

associated with the lipid in the sample.

10.   The method of claim 1, wherein the apolipoprotein is selected from

the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

11.   The method of claim 1, wherein the biological sample is selected from

the group consisting of blood, plasma, and serum.

12.   (four times amended) A method of determining the relative

concentration of at least two different apolipoproteins in a biological sample

comprising:

mixing in solution a first and second monoclonal antibody molecules each

immunoreactive with a specific different apolipoprotein into the sample, wherein at

least one of the first and second monoclonal antibodies bind to a stable,

conformation independent epitope of a lipoprotein that is uninfluenced by the lipid

content of the lipoprotein, protein component of the lipoprotein or lipid associated

with the specific lipoprotein in a conformation and lipid content independent

manner;

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allowing the monoclonal antibody molecules to bind to the apolipoproteins in the sample,

immersing into the mixture third immobilized monoclonal antibody molecules immunoreactive with a second, distinct epitope of one of the first or second apolipoproteins,

allowing the third immobilized monoclonal antibody molecules to bind to one of the apolipoproteins bound by either the first or second monoclonal antibodies,

determining the amount of apolipoprotein bound by the first and second monoclonal antibodies and the amount of protein bound by the third immobilized monoclonal antibodies, and

subtracting from the total apolipoprotein bound by the first and second monoclonal antibodies the amount of protein bound by the third immobilized monoclonal antibodies, to yield the amounts of the first and second apolipoproteins .

13.   (amended) The method of claim 12 wherein the apolipoprotein bound by one of the monoclonal antibodies in solution is apolipoprotein Apo B-100.

39.   (three times amended) A method for determining the relative ratio of LDL to HDL in a biological sample comprising

(a) determining the amount of LDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with low density lipoprotein and not cross-reactive with high density lipoprotein and determining the amount of low density lipoprotein;

(b) determining the amount of HDL in the sample by

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adding to the sample monoclonal antibody molecules immunoreactive with

high density lipoprotein and not cross-reactive with low density lipoprotein and

determining the amount of high density lipoprotein; and

(c) determining the ratio of the amount of low density lipoprotein with the

amount of high density lipoprotein, wherein at least one of the monoclonal

antibodies to LDL and HDL bind a stable, conformation independent epitope that is

uninfluenced by the lipid content of the lipoprotein, the protein component of the

lipoprotein or lipid associated with the specific lipoprotein.

40.   (twice amended) A method for determining the relative ratio of VLDL

to HDL in a biological sample comprising

(a) determining the amount of VLDL in the sample by

determining the amount of Apo C-III present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and

high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

contacting the anti-ApoC-III antibody reactive with Apo C-III with the

biological sample to form complexes between the anti-ApoC-III antibody and the

Apo C-III containing lipoprotein particles,

contacting the Pan B antibody with the biological sample containing the anti-

ApoC-III antibody bound to the Apo C-III containing lipoprotein particles,

separating the complexed Pan B-anti-ApoC-III antibody-lipoprotein particles

from the biological sample, and

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determining the amount of complexed Pan B-anti-ApoC-III antibody-

lipoprotein particles, which is the amount of Apo C-III present in VLDL in the anti-

Apo C-III anti-Apo B complexed material in the sample;

and

(b) determining the amount of HDL in the sample by

determining the amount of Apo C-III present in the HDL in the sample by

providing Apo A-I monoclonal antibody specifically immunoreactive with Apo

A-I,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

contacting the antibody reactive with Apo C-III with the biological sample to

form complexes between the anti-Apo C-III antibody and the Apo C-III containing

lipoprotein particles,

contacting the anti-Apo A-I antibody with the biological sample to form

complexes with the anti-Apo C-III antibody-Apo C-III containing lipoprotein

particles,

separating the complexed anti-Apo C-III antibody-Apo C-III containing

lipoprotein particles from the biological sample,

determining the amount of Apo C-III present in HDL in the anti-Apo C-III-

anti-Apo A-I complexed material in the sample, and

determining the ratio of Apo C-III present in VLDL in the sample to Apo C-

III present in HDL in the sample, which is the ratio of VLDL to HDL,

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wherein the VLDL and HDL are measured in the same sample using

immobilized anti-Apo A-I and anti-Apo B or anti-Apo C-III antibodies or measured

by immunoprecipitation with the anti-Apo A-I and anti-ApoB antibodies or anti-Apo

C-III antibodies in separate samples,

wherein at least one of the monoclonal antibodies bind to a stable,

conformation independent epitope that is uninfluenced by the lipid content of the

lipoprotein, apolipoprotein or lipid associated with a specific lipoprotein selected

from the group consisting of Apo AI, Apo B, and Apo CIII.

41.   (three times amended) A method for determining the relative ratio of

VLDL to HDL comprising

(a) determining the amount of VLDL in the sample by

determining the amount of Apo E present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and

high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody which specifically binds to Apo E associated

with VLDL,

contacting the antibodies reactive with Apo E associated with VLDL with the

biological sample to form complexes between the anti-ApoE antibodies and Apo E

containing particles,

contacting Pan B antibody with the biological sample containing the

complexes between the anti-ApoE antibodies and ApoE containing particles to form

complexes of anti-ApoB-anti-ApoE-ApoE containing particles, and

determining the amount of Apo E in the complexes of anti-ApoB-anti-ApoE-ApoE containing particles, which is the Apo E present in VLDL in the sample;

(b) removing the complexes of anti-ApoB-anti-ApoE-ApoE containing particles, either by binding of the anti-Apo E antibodies to an immobilized surface or centrifugation of sample to remove the complexes of anti-ApoB-anti-ApoE-ApoE containing particles;

and

(c) determining the amount of HDL in the sample by

determining the amount of Apo E present in the HDL in the sample by

providing Apo A-I monoclonal antibody immunoreactive specifically with Apo A-I,

contacting antibodies reactive with Apo E in HDL particles with the biological sample to form complexes between the anti-ApoE antibodies and Apo E containing particles,

contacting the Apo A-I monoclonal antibody with the biological sample to form complexes of the anti-ApoE antibodies-ApoE containing particles-anti-ApoA-I,

determining the amount of Apo E present in HDL in the complexes of the anti-ApoE antibodies-ApoE containing particles-anti-Apo A-I in the sample, and

determining the ratio of Apo E present in VLDL in the sample and Apo E present in HDL in the sample which is the ratio of VLDL to HDL,

wherein at least one of the monoclonal antibodies bind to a stable, conformation independent epitope that is uninfluenced by the lipid content of the

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lipoprotein, protein component of the lipoprotein or lipid associated with a specific

lipoprotein selected from the group consisting of Apo B, Apo AI, and Apo E.

42.   (three times amended) A kit for determining the relative ratio of

VLDL to HDL comprising

Pan B antibody which is characterized by an equal binding and high affinity

for all Apo B-containing lipoproteins in human plasma,

monoclonal or recombinant antibody specifically immunoreactive with Apo C-

III, and

monoclonal or recombinant Apo A-I antibody specifically immunoreactive

with Apo A-I,

wherein at least one of the monoclonal or recombinant antibodies specifically

bind to a stable, conformation independent epitope of a lipoprotein including Apo C-

III or Apo A-I that is uninfluenced by the lipid content of the lipoprotein, protein

component thereof or lipid associated with a specific lipoprotein selected from the

group consisting of Apo AI, and Apo CIII.

43.   (twice amended) The kit of claim 42 wherein the anti-Apo C-III or

anti-A-1 monoclonal or recombinant antibody molecules are selected from the group

consisting of monoclonal antibodies, recombinant antibodies, and antigen binding

antibody fragments thereof that specifically bind to a stable, conformation

independent epitope which is uninfluenced by the lipid content of the lipoprotein,

protein component thereof, or lipid associated with a specific lipoprotein.

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44.   (twice amended) A kit for determining the relative ratio of VLDL to

HDL comprising

Pan B antibody which is characterized by an equal binding and high affinity

for all Apo B-containing lipoproteins in human plasma,

monoclonal antibody which predominantly binds to Apo E associated with

VLDL ,

monoclonal Apo A-I antibody specifically immunoreactive with Apo A-I, and

monoclonal antibody which predominantly binds to Apo E in HDL,

wherein at least one of the antibodies binds to a stable, conformation

independent epitope of a lipoprotein containing Apo E or Apo A-I that is

uninfluenced by the lipid content of the lipoprotein, protein component of the

lipoprotein or lipid associated with a specific lipoprotein.

46.   (twice amended) A kit for determining the relative ratio of LPA-I and

LPA-II lipoprotein particles comprising

monoclonal or recombinant Apo-A-I antibody specifically immunoreactive

with Apo A-I lipoproteins in human plasma; and

monoclonal or recombinant Apo A-II antibody specifically immunoreactive

with Apo A-II,

wherein the anti-Apo A-I or anti-Apo A-II monoclonal or recombinant

antibody molecules are selected from the group consisting of monoclonal antibodies,

recombinant antibodies, and antigen-binding antibody fragments thereof that

specifically bind to a stable, conformation independent epitope of a lipoprotein

containing Apo A-I or Apo A-II which is uninfluenced by the lipid content of the

lipoprotein, protein component of the lipoprotein, or lipid associated with a specific

lipoprotein.

47. (twice amended) The kit of claim 46 wherein the anti-Apo A-I and

anti-Apo A-II monoclonal or recombinant antibody molecules are selected from the

group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal

antibody fragments that specifically bind to a stable, conformation independent

epitope which is uninfluenced by the lipid content of the lipoprotein, protein

component of the lipoprotein, or lipid associated with a specific lipoprotein.

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